Summary of Chapter 4

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   4.12 Myoglobin and Hemoglobin
   4.13 Oxygen Binding to Myoglobin and Hemoglobin
Chapter 4 Proteins: Three Dimensional Structure and Function

**Conformation** - three dimensional shape

*NATIVE conformation* - each protein folds into a single stable shape (physiological conditions)

**Biological function ↔ native conformation**

**Types of Proteins**
- Fibrous – Collagen
- Water Soluble – Myoglobin
- Membrane – Bacteriorhopsin (Chapter 9)

Proteomics – Study of Dynamic Protein Expression and Relation to Cell Metabolism

*4000 E. coli proteins*
2D PAGE Gels
Excise Spots
Trypsin Digestion
MS of Fragments
Protein Identification
4.1 There Are Four Levels of Protein Structure

- **Primary structure** - amino acid linear sequence
- **Secondary structure** – sequences with regions of regularly repeating conformations (α-helices; β-sheets; β-turns; unordered)
- **Tertiary structure** – 3D structure of a single linear sequences (spatial arrangement of secondary structures)
- **Quaternary structure** – spatial arrangement of 3D structures of two or more polypeptide chains
4.2 Methods for Determining Protein Structure

X-ray crystallography requires a single crystal that diffracts x-rays to determine 3D structure

![Diagram of X-ray crystallography]

Structural Representations

Computer Programs are used to analyze the diffraction pattern → 3D Structures

**Fig 4.3 Ribonuclease A**

(a) Space-filling model  
(bound substrate analog black)

(b) Cartoon ribbon model  
(shows secondary structure)

(c) Substrate-binding site view
NMR determination of protein structure

- Nuclear Magnetic Resonance
- Solution studies
- Labeled Proteins ($^{13}\text{C}; ^{15}\text{N}$)
- Constraints (NOE; J-coupling)

Computer Methods

Fig 4.4 Ribonuclease A
Several NMR structures are overlaid

4.3 The Conformation of the Peptide Group

- Peptide bond properties
- Planar (resonance)
- H-bond Donor (N-H)
- H-bond Acceptor (O-)

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Fig. 4.7 *Trans* and *cis* conformations of a peptide group

*trans* conformation favored (less steric interactions)

Rotation of atoms in a peptide group

Bond Rotation occurs about both the $N-C_\alpha(\phi)$ and $C_\alpha-C(\Psi)$ bonds (in the $N-C_\alpha-C$ backbone)

Rotation of the $N-C_\alpha$ bond in proline is restricted because of the pyrrolidine ring structure
Steric Interactions Prevent Many ($\Phi$, $\Psi$) Angles

($\Phi$, $\Psi$) Angles near to $0^\circ$ are forbidden due to severe steric hinderance

Fig. 4.9 (a) Ramachandran Plot

- Ramachandran plots
  - Energy Contour
  Plots of $\phi$ and $\psi$
  (blue low energy)
- Secondary structures fall within permissible areas
**Fig. 4.9 (b) Ramachandran Plot**

Observed $\phi$ and $\psi$ values in known structures fall in **Low Energy** area.

Secondary structures are **grouped** within permissible areas: 
- $\alpha$-Helix residues (red),
- $\beta$-Strand residues (blue),
- others (green).

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**4.4 The $\alpha$-Helix**

- Right-handed rotations of 100° about a cylinder axis and translations of 0.15 nm along the cylinder axis
- The $\phi$ and $\psi$ angles of each residue are similar: near $-57^\circ$ ($\phi$) and near $-47^\circ$ ($\psi$)
- Each C=O (residue $n$) forms a hydrogen bond with the amide hydrogen (N-H) of residue $n+4$
- Helix is stabilized by many hydrogen bonds (which are nearly parallel to long axis of the helix)
- All C=O groups point toward the C-terminus (entire helix is a dipole with (+) N, (-) C-termini)
Amide Bond Geometry in an Alpha Helix

- Ala--Val--Leu--Ser--Lys-
  \[ \text{n} \quad \text{n+1} \quad \text{n+2} \quad \text{n+3} \quad \text{n+4} \]

\( \text{C=O of Ala (n+0)} \)
H-bonded to
\( \text{N-H of Lys (n+4)} \)

Three rotations
\[ 3 \times 100^\circ = 300^\circ \]
Three translations
\[ 3 \times 0.15 \text{ nm} = 0.45 \text{ nm} \]

Properties of the alpha helix (cont)

• **Residues per turn** (1 residue/100° = 3.6 residues/360° turn)

• **Rise** – Distance along the axis of the helix between residues (0.15 nm)

• **Pitch** is recurrence of equivalent positions (0.54 nm = 3.6 residues/turn x 0.15 nm/residue)

• **Chiral** - Most \( \alpha \) helices in proteins are **right** handed (backbone turns clockwise when viewed along the axis from the N terminus)
Fig. 4.10
The $\alpha$-helix

Fig. 4.11 Stereo view of right-handed $\alpha$ helix

- All side chains project outward from helix axis
4.5 β Strands and β Sheets

**β Strands** - polypeptide chains that are almost fully extended with $(\Phi, \Psi)$ in upper left quadrant of Ramachandran Plot

**β Sheets** - multiple β strands arranged side-by-side

Parallel (strands run in same direction) or Antiparallel (strands run in opposite direction) β Sheets are stabilized by hydrogen bonds between C=O and -NH
Fig 4.15 β-Sheets (a) parallel, (b) antiparallel

Fig 4.16 Stereo view of antiparallel β sheet

Pleated Sheet

Hydrophobic

Hydrophilic
Interactions of $\beta$ sheets

Hydrophobic side chains of one face of a sheet can interact with other hydrophobic residues in protein interior (e.g., second sheet; amphipathic $\alpha$ helices)

4.6 Loops and Turns

Nonrepetitive secondary structures

Loops - often contain hydrophilic residues and are found on protein surfaces

Other primary sequences within a protein can also be nonrepetitive in secondary structure

Regular Secondary Structures

$\beta$ Turns (reverse turns) - loops containing 5 residues or less that are stabilized by hydrogen bonding
4.7 Tertiary Structure of Proteins

• Three-dimensional structures allowing secondary structures (amino acids far apart in the primary structure) to be folded together

• Stabilized primarily by noncovalent interactions between side chains and by disulfide bridges (if present)

• Subclasses of Protein Structures/Protein Folds
  Alpha Structures; Alpha/Beta Structures; (Antiparallel) Beta Structures; Irregular Structures
A. Supersecondary Structures (Motifs)

- Helix-loop-helix
- Helix bundle
- βαβ unit
- Coiled coil
- Helix Dipole
- Hydrophobic Contacts
- Helix on Exterior

Supersecondary structures (beta)

- Hairpin
- β meander
- β-sandwich
- Greek key
B. Domains

Independently folded, compact units in proteins

Domains are connected to each other by loops or by weak noncovalent contacts to other domains

All $\alpha$ - $\alpha$ helix motifs with loops

All $\beta$ - $\beta$ sheet motifs with loops

Mixed $\alpha/\beta$ – beta-alpha-beta motif with loops

$\alpha + \beta$ – local clusters of alpha and beta motifs in a tightly packed unit with loops

Cytochrome c: High Structure and Sequence Homology

(a) Tuna (+heme)
(b) Tuna
(c) Rice
(d) Yeast
(e) Bacteria
High Structural Homology with Little Sequence Homology
(a) lactate dehydrogenase, (b) malate dehydrogenase

23% Sequence Homology

Lactate/Malate Dehydrogenases Catalyze Similar Reactions (Chpt 13)

Krebs Cycle

L-Malate

Oxaloacetate

L-Lactate

Pyruvate
Fig 4.24 Examples of tertiary structure

All-alpha
Binds FAs

All-beta
Binds carbohydrates

(a)

Human serum albumin

(d)

Jack bean concanavalin A

Tertiary protein structures (cont)

Alpha/beta
Reducing Agent

Alpha + beta
Surface Protein

Human thioredoxin

Neisseria gonorrhoea pilin
4.8 Quaternary Structure

- Refers to the organization of subunits in a protein with multiple subunits (an “oligomer”)

- Subunits (may be identical or different) have a defined stoichiometry and arrangement
  - $\alpha\alpha$ (homodimer); $\alpha\beta$ (heterodimer); $\alpha\beta\gamma_2$ (tetramer)

- Subunits are held together by many weak, noncovalent interactions (hydrophobic, electrostatic)

**Fig 4.25 Quaternary structure of multidomain proteins**

(a) Chicken triose phosphate isomerase  
(b) Bacteriophage MS2 capsid protein

**Homodimer $\alpha_2$**  
**Trimer $\alpha_3$**
4.9 Protein Denaturation and Renaturation

- **Denaturation** - disruption of native conformation of a protein, with loss of biological activity
- **Renaturation** – refolding of biological active conformation

\[ F \rightleftharpoons U \quad Keq = \frac{[U]}{[F]} \quad \Delta G = -RT\ln Keq \]

- Native State is marginally stable over Unfolded State (0.4 kJ/mol-residue $\Rightarrow$ 40 kJ/100 residues)
- Proteins denatured by heating, pH or chemicals like urea or guanidinium chloride $\Delta S$ increases

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**Thermal Unfolding**

**Ribonuclease A**

$T_m$ is the midpoint of unfolding ($[N] = [U]$)

$T_m \Rightarrow \Delta G$

Unfolding is cooperative (curve sigmodial)

Single Domain Proteins unfold with a single sigmodial curve

UV (blue), viscosity (red), optical rotation (green)
Urea and guanidinium chloride are chaotropic agents used to study protein stability

\[
\begin{align*}
\text{Urea} & \quad \text{Increasing [Urea] or [GdnHCl]} \\
\text{Guanidinium chloride} & \quad \text{leads to cooperative protein unfolding like increasing temperature.}
\end{align*}
\]

Water interacts better with hydrophobic groups when chaotropic agents are present (lessen the hydrophobic effect)

\[
\text{[Denaturant]} = 50\% \quad \text{Unfolding is related to } \Delta G \text{ stability of } N
\]

Denaturation and renaturation of ribonuclease A

Inactive RNase A

With incorrectly formed Disulfides spontaneously

Refolds to N
4.10 *In vivo* Protein Folding and Stability

N is a single low-energy structure.

Many structures compose U (i.e. A and B).

U $\rightarrow$ N is rapid, spontaneous and cooperative.

$1^\circ \rightarrow 2^\circ \rightarrow 3^\circ$

Folding Code not unraveled (yet)

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**Transient Intermediates Aid Protein Folding**

U $\rightarrow$ I $\rightarrow$ N occurs rapidly (<10 s).

Intermediates (I)

*hydrophobic collapse*

*molten globule*

(native-like 2° but not 3° structure)

Many folding pathways occur.
A. The Hydrophobic Effect

Nonpolar side chains associate and exclude water causing a collapse to a molten globule (\(\Delta S > 0\) from release of solvated water to bulk)

Secondary Structure can form without water present (competition for H-bonds)

Molten globule secondary structure is usually native-like and determined by amino acid sequence

B. Hydrogen Bonding

Contributes to cooperativity of folding

H-bonds stabilize: secondary structures and tertiary structures

<table>
<thead>
<tr>
<th>Type of hydrogen bond</th>
<th>Typical distance between donor and acceptor atom (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyl-hydroxyl</td>
<td>0.28</td>
</tr>
<tr>
<td>Hydroxyl-carbonyl</td>
<td>0.28</td>
</tr>
<tr>
<td>Amide-carbonyl</td>
<td>0.29</td>
</tr>
<tr>
<td>Amide-hydroxyl</td>
<td>0.30</td>
</tr>
<tr>
<td>Amide-imidazole nitrogen</td>
<td>0.31</td>
</tr>
</tbody>
</table>
C. Van der Waals and Charge-Charge Interactions

VDW contacts occur between nonpolar side chains and contribute to the stability of proteins.

Charge-charge interactions between oppositely charged side chains in the interior of a protein also stabilize protein structure.

A Native State is Stabilized by Entropy and Enthalpy Changes from Nonpolar Groups excluded from water

\[ U \rightleftharpoons F; \quad K_{eq} = [F]/[U] \]
\[ \Delta G = -RT \ln K_{eq} < 0 \]
\[ \Delta G = G_f - G_u = \Delta G_{\text{chain}} + \Delta G_{\text{solvent}} \]

Interactions between the peptide chain and water are favorable for folding (driven by a large negative \(-T \Delta S_{\text{solvent}}\))

A folded chain is highly ordered which does not favor folding (since \(-T \Delta S_{\text{chain}}\) is positive)
D. Protein Folding Is Assisted by Chaperones

Molecular chaperones or heat shock proteins increase rate of correct folding (prevent incorrectly folded intermediates)

Chaperones bind to unfolded protein and hydrolyze ATP to refold U to F

4.11 Collagen, a Fibrous Protein

- Collagen is a major structural protein in vertebrates (25-35% of total protein in mammals)
- Diverse forms/types in tendons (ropelike fibers), skin (loosely woven fibers), bone, cartilage in joints
- Tropocollagen is the basic building block and it consists of three left-handed helical chains coiled around each other in a right-handed supercoil (Mr = 285 kDa; 1000 amino acid residues; 1.5 x 300 nm)
- Triple Helix has three amino acids per turn, rise 0.31 nm per residue (more extended than an α helix)
**Tropocollagen triple helix**

Repeating \(-\text{Gly-X-Y-}\); where \(X\) is often proline and \(Y\) is often 4-hydroxyproline

Glycine is near helical axis
(other residues cannot fit)

Gly

\[
\begin{array}{c}
\text{Gly} \\
\circ\text{ (helical axis)} \\
\text{Gly}
\end{array}
\]

**Interchain H bonding in tropocollagen**

- Each Amide N-H of Gly in one chain is H-bonded to C=O in another chain
- No Intrachain H-bonds exist
4-Hydroxyproline and 5-hydroxylysine

Formed by enzyme hydroxylation reactions (require vitamin C) after incorporation into collagen (-OH often linked to Glc-Gal-)

Covalent cross-links in tropocollagen

Enzymatic Rx:
Lys to Allylysine
Collagen Fibrils are Rigid in Bone

Tropocollagen ‘Bricks’ 1.5 x ~300 nm
~40-nm holes and ~64-nm striations
Covalent cross links and Bone Mineral Content (Ca₃(PO₄)₂)
High Tensile Strength (greater than steel wire)

4.12 Structures of Myoglobin and Hemoglobin

- **Myoglobin (Mb)** - oxygen to muscle
- **Hemoglobin (Hb)** - carries oxygen in the blood
- **Heme** tetrapyrrole rings complexed with iron Fe(II)-protoporphyrin IX
**Protein component of Mb and Hb is globin**

8 α helices (all-alpha)

Interior hydrophobic

Heme **prosthetic group** binds to **hydrophobic** cleft

Heme Fe(II) binds **oxygen** and **His-93** (His-64 H-bonds with oxygen)

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**Hemoglobin (Hb)**

α<sub>2</sub>β<sub>2</sub> tetramer

α chain - 7 α helices

β chain - 8 α helices

3D structure of subunits similar to Mb

4 heme groups
Fig 4.43 Tertiary structure of myoglobin, α-globin and β-globin

α-Globin (blue)
β-Globin (purple)
Myoglobin (green)

4.13 Oxygen Binding to Mb and Hb

Deoxymyoglobin; Oxymyoglobin

His-64 (distal) and His-93 (proximal)
B. Oxygen-Binding Curves of Mb and Hb

**Mb + O_2 ⇌ MbO_2**

*No cooperativity (sigmoid)*

\[ K_a = \frac{[MbO_2]}{[Mb][O_2]} \]

\[ Y = \frac{[Bound]}{[Total]} \]

\[ Y = \frac{[O_2]}{[O_2] + K_d} \]

\[ K_d = \frac{1}{K_a} \leftrightarrow P_{50} \]

Small \( P_{50} \) implies tight binding.

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**Hb + 4O_2 ⇌ HbO_2**

*Positive cooperativity (hyperbolic)*

\[ K_a = \frac{[HbO_2]}{[Hb][O_2]^4} \]

\[ Y = \frac{[Bound]}{[Total]} \]

\[ Y = \frac{[O_2]^4}{[O_2]^4 + K_d} \]

\[ K_d = \frac{1}{K_a} \leftrightarrow P_{50} \]
Model for Cooperative Oxygen-binding to Hb

**R-state** (tight)

**T-state** (weak)

Each sigmoidal

Switching from **T** to **R** as **O₂** increases yields **Hb** curve and positive cooperativity

Advantages?

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**Fig 4.47 Conformational changes in a hemoglobin chain induced by oxygenation**

- Oxygen binding to Fe pulls the proximal His toward heme plane
- Helix with His shifts position (blue to red position) (3° change)
- Ion pairs between subunits of Hb disrupted (4° change)
- Fe(II) in another subunit is closer to Heme plane and binds oxygen better
C. Hemoglobin is an Allosteric Protein

Allosteric binding – binding of L to one site (subunit) affects binding of the another site

Allosteric effectors (modulators) – ligands that bind to protein sites separate from the functional binding site (may be activators or inhibitors)

Activators increase population of R-state
Inhibitors increase population of the T-state

2,3 Bisphospho-D-glycerate (2,3BPG) is an allosteric inhibitor of Hb

2,3BPG lowers the affinity of deoxyHb for oxygen (P<sub>50</sub> ~12 to ~26 torr)
Oxidative Metabolites ($H^+$ and $CO_2$) Decrease Affinity of Oxygen for Hb

**Bohr Effect** - Lowering pH occurs with increasing CO2 and stabilizes T-state

**Carbamate Adducts** – Increasing CO2 blocks NH2-groups of subunits (T-state stabilized)

![Graph showing pH vs. $pO_2$ with different pH values and structures depicting carbamate adducts]